

AMENDMENT TO THE CLAIMS:

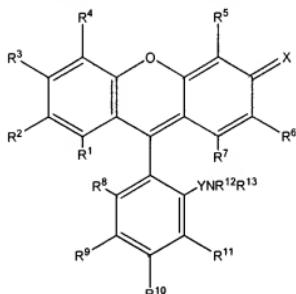
This listing of claims will replace all prior versions, and listings of claims in the application:

LISTING OF CLAIMS:

The list of currently pending claims is presented below.

WHAT IS CLAIMED IS:

- 1 1. (Original) A xanthene dye having the formula:



2

- 3 in which

4 R¹, R², R⁴, R⁵, R⁶, R⁷, R⁸, R⁹, R¹⁰ and R¹¹ are independently selected from substituted or
5 unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or
6 unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or
7 unsubstituted heterocycloalkyl, halogen, H, NO₂, CN and C(Z')R¹⁴, NR¹⁵R¹⁶ and
8 Z²R¹⁶;

9 R³ is selected from Z²R¹⁶ and NR¹⁵R¹⁶

10 wherein

11 Z¹ is a member selected from O, S and NH;

12 Z² is a member selected from O and S;

13 R¹⁵ is a member selected from H, substituted or unsubstituted alkyl, and
14 substituted or unsubstituted heteroalkyl;

15 R¹⁶ is selected from H, substituted or unsubstituted alkyl, substituted or
16 unsubstituted heteroalkyl, C(Z³)R¹⁷, and a nitrogen-containing reactive
17 group comprising R¹⁵ and R¹⁶, together with the nitrogen to which they are
18 attached, wherein said reactive group is a member selected from -NHNH₂,
19 -N=C=S and -N=C=O

20 wherein

21 Z³ is a member selected from O, S and NH;

22 R¹⁷ is a member selected from substituted or unsubstituted alkyl,
23 substituted or unsubstituted heteroalkyl, OR¹⁸, and NR¹⁹R²⁰

24 wherein

25 R¹⁸ is a member selected from H, substituted or unsubstituted
26 alkyl, substituted or unsubstituted heteroalkyl, substituted or
27 unsubstituted aryl, substituted or unsubstituted
28 heteroaryl and C(O)R²¹

29 wherein

30 R²¹ is substituted or unsubstituted alkyl or substituted or
31 unsubstituted heteroalkyl;

32 R¹⁹ and R²⁰ are members independently selected from H,
33 substituted or unsubstituted alkyl and substituted or
34 unsubstituted heteroalkyl

35 Y is a member selected from C(O) and S(O)₂;

36 X is a member selected from (NR²²R²³) and (O)

37 wherein

38 R²² and R²³ are members independently selected from H, substituted or
39 unsubstituted alkyl and substituted or unsubstituted heteroalkyl; and

40 R¹² and R¹³ are members independently selected from substituted or unsubstituted alkyl,
41 substituted or unsubstituted heteroalkyl, substituted or unsubstituted
42 heterocycloalkyl, substituted or unsubstituted aryl and substituted or unsubstituted
43 heteroaryl, with the proviso that at least one of R¹² or R¹³ comprises a member
44 selected from a bond to a carrier molecule, a bond to a linker bound to a carrier
45 molecule, a bond to a solid support, a bond to a linker attached to a solid support,
46 a bond to a fluorescence quencher, a bond to a linker to a fluorescence quencher
47 and an oxygen-containing reactive group, and further with the proviso that when
48 R¹² and R¹³, together with the nitrogen to which they are attached form a
49 piperazine ring said oxygen-containing reactive group is a phosphoramidite and
50 said bond to a carrier molecule is other than a bond to a peptide.

1 2. (Original) The xanthene dye according to claim 1, wherein R³ is R¹⁵R¹⁶N; and X
2 is NR²³R²⁴, wherein R¹⁵, R¹⁶, R²³ and R²⁴ are members independently selected from H,
3 substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl.

1 3. (Original) The xanthene dye according to claim 1, wherein at least one of R⁸, R⁹,
2 R¹⁰ and R¹¹ is a halogen.

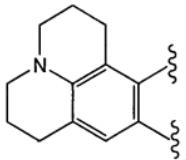
1 4. (Original) The xanthene dye according to claim 1, wherein R⁹ and R¹⁰ are
2 halogen.

1 5. (Original) The xanthene dye according to claim 3, wherein R³ is OR¹⁶; and X is
2 O.

1 6. (Original) The xanthene dye according to claim 5, wherein R² and R⁶ are
2 halogen.

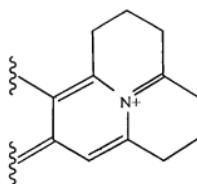
1 7. (Original) The xanthene dye according to claim 5, wherein R² and R⁶ are
2 independently selected from substituted or unsubstituted alkyl and substituted or unsubstituted
3 heteroalkyl.

1 8. (Original) The xanthene dye according to claim 1, wherein R³ is NR¹⁵R¹⁶ and R²,
2 R⁴ and R¹⁵ and R¹⁶, together with the nitrogen atom to which they are bound, are fused with the
3 phenyl moiety to which NR¹⁵R¹⁶, R² and R⁴ are bound, forming a substituted or unsubstituted
4 ring system having formula:



5

1 9. (Original) The xanthene dye according to claim 1, wherein X is NR²²R²³ and R⁵,
2 R⁶ and R²² and R²³, together with the nitrogen atom to which they are bound, are fused with the
3 unsaturated 6-member ring to which NR²²R²³, R⁵ and R⁶ are bound, forming a substituted or
4 unsubstituted ring system having the formula:

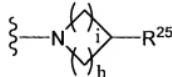


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1 10. (Original) The xanthene dye according to claim 1, wherein said oxygen-
2 containing reactive functional group is a member selected from hydroxyl and activated
3 derivatives thereof, phosphoramidite, and carboxylic acid and activated derivatives thereof.

1 11. (Original) The xanthene dye according to claim 1, wherein R¹² and R¹³, together
2 with the nitrogen to which they are bound are joined to form a ring system.

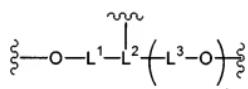
1 **12.** (Original) The xanthene dye according to claim 11, wherein NR¹²R¹³ has the
2 formula:



3 wherein

4 h and i are members independently selected from integers such that the sum (h + i) is
5 from 4-8; and
6 R²⁵ is a reactive functional group.

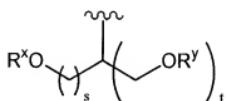
1 **13.** (Original) The xanthene dye according to claim 1, wherein R¹² comprises a
2 moiety having the formula:



3 wherein

4 L¹, L² and L³ are members independently selected from substituted or
5 unsubstituted alkyl and substituted or unsubstituted heteroalkyl; and
6 t is 0 or 1.

1 **14.** (Original) The xanthene dye according to claim 13, said moiety having the
2 formula:



3 wherein

4 R^x and R^y are members independently selected from H, substituted or
5 unsubstituted alkyl, substituted or unsubstituted heteroalkyl, a hydroxyl-

protecting group, a phosphate moiety, a phosphodiester moiety, a phosphorus-containing internucleotide bridge of a nucleic acid, a solid support, a carrier molecule and $-\text{OP}(\text{O})(\text{OR}')_2(\text{N}(\text{R}'\text{R}'')_2$

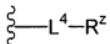
wherein

R⁰, R^p and R^d are members independently selected from H, substituted or unsubstituted C₁-C₆ alkyl and substituted or unsubstituted C₁-C₆ heteroalkyl; and

s is an integer from 1 to 20.

15. (Original) The xanthene dye according to claim 14, wherein R^o is CH_2CH_2CN .

16. (Original) The xanthene dye according to claim 14, wherein at least one of R^x and R^y comprises a moiety having the formula:

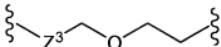


wherein

L^4 is a member selected from a bond, substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl; and

R^z is a member selected from a reactive functional group, solid support, a nucleic acid, a saccharide and a peptide.

17. (Original) The xanthene dye according to claim 16, wherein L⁴ comprises a moiety having the formula:



wherein Z³ is a member selected from CH₂ and C≡O.

18. (Original) The xanthene dye according to claim 1, wherein said carrier molecule
further comprises a quencher moiety.

1 **19.** (Original) The xanthene dye according to claim **18**, wherein said xanthene dye
2 and said quencher comprise a donor-acceptor energy transfer pair.

1 **20.** (Original) The xanthene dye according to claim **18**, wherein said quencher has
2 substantially no native fluorescence.

1 **21.** (Original) The xanthene dye according to claim **20**, wherein said quencher
2 comprises at least three residues selected from aryl, substituted aryl, heteroaryl, substituted
3 heteroaryl and combinations thereof, wherein at least two of said residues are covalently linked
4 via an exocyclic diazo bond.

1 **22.** (Original) The xanthene dye according to claim **1**, wherein said xanthene dye is
2 attached to a nucleic acid at a position which is a member selected from the 3'-terminus, the 5'-
3 terminus, a nucleobase, and a phosphorus-containing internucleotide bridge of said nucleic acid.

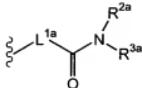
1 **23.** (Original) The xanthene dye according to claim **18**, wherein said nucleic acid is a
2 probe which is a member selected from molecular beacons, scorpion probes, sunrise probes,
3 conformationally assisted probes and TaqMan™ probes.

1 **24.** (Original) The xanthene dye according to claim **1**, wherein said carrier molecule
2 is a peptide comprising a cleavage recognition site for an enzyme.

1 **25.** (Original) The xanthene dye according to claim **24**, wherein said peptide
2 comprises a cleavage recognition site for a protease.

1 **26.** (Original) The xanthene dye according to claim **24**, wherein said cleavage
2 recognition site is for an enzyme selected from trypsin, enterokinase, HIV-1 protease,
3 prohormone convertase, interleukin-1b-converting enzyme, adenovirus endopeptidase,
4 cytomegalovirus assemblin, leishmanolysin, β-secretase for amyloid precursor protein, thrombin,
5 renin, angiotensin-converting enzyme, cathepsin-D and a kininogenase.

1 **27.** (Original) The xanthene dye according to claim **1**, in which R¹² has the formula:



2

3 wherein

4 L^{1a} is a member selected from substituted or unsubstituted alkyl, and substituted
5 or unsubstituted heteroalkyl groups; and

6 R^{2a} and R^{3a} are members independently selected from H, substituted or
7 unsubstituted alkyl, and substituted or unsubstituted heteroalkyl, and R²
8 and R³, together with the nitrogen to which they are attached, are
9 optionally joined to form a ring which is a member selected from
10 substituted or unsubstituted C₅-C₇ cycloalkyl and substituted or
11 unsubstituted 5-7-membered heterocycloalkyl.

1 **28.** (Original) The xanthene dye according to claim 27, in which L^{1a} does not
2 comprise a member selected from a carboxylic acid and a carboxylic acid ester.

1 **29.** (Original) A method for determining whether a sample contains an enzyme, said
2 method comprising:

3 (a) contacting said sample with a peptide construct comprising:
4 i) a xanthene dye according to claim 1;
5 ii) a quencher; and
6 iii) a cleavage recognition site for said enzyme,

7 wherein said peptide is in a conformation allowing donor-acceptor energy transfer
8 between said fluorophore and said quencher when said fluorophore is
9 excited;

10 (b) exciting said xanthene dye; and
11 (c) determining a fluorescence property of said sample, wherein the presence of said
12 enzyme in said sample results in a change in said fluorescence property.

1 **30.** (Original) A method for determining whether a compound alters an activity of an
2 enzyme, said method comprising:

- 3 (a) contacting a sample comprising said enzyme and said compound with a peptide
4 construct comprising:
5 i) a xanthene dye according to claim 1;
6 ii) a quencher; and
7 iii) a cleavage recognition site for said enzyme,
8 wherein said peptide is in a conformation allowing donor-acceptor energy transfer
9 between said xanthene dye and said quencher when said xanthene dye is
10 excited;
11 (b) exciting said xanthene dye; and
12 (c) determining a fluorescence property of said sample, wherein said activity of said
13 enzyme in said sample results in a change in said fluorescence property.

1 **31.** (Original) A method for detecting a nucleic acid target sequence, said method
2 comprising:

- 3 (a) contacting said target sequence with a detector oligonucleotide comprising a target
4 binding sequence, said detector oligonucleotide having linked thereto,
5 i) a xanthene dye according to claim 1; and
6 ii) a quencher,
7 wherein said detector nucleic acid is in a conformation allowing donor-acceptor
8 energy transfer between said xanthene dye and said quencher when said
9 xanthene dye is excited;
10 (b) hybridizing said target binding sequence to said single-stranded target sequence,
11 thereby altering said conformation of said detector oligonucleotide, causing a change
12 in a fluorescence parameter; and

13 (c) detecting said change in said fluorescence parameter, thereby detecting said nucleic
14 acid target sequence.

1 **32.** (Original) The method according to claim 31, wherein said complementary strand
2 is synthesized in a target amplification reaction.

1 **33.** (Original) The method according to claim 31, wherein said complementary strand
2 is synthesized by extension of the target sequence using said detector oligonucleotide as a
3 template.

1 **34.** (Currently Amended) The method according to claim 31, wherein said
2 fluorescence parameter is detected in [[--]]real-time.

1 **35.** (Original) A method for detecting amplification of a target sequence comprising,
2 in an amplification reaction:

3 (a) hybridizing to said target sequence a detector oligonucleotide comprising a single-
4 stranded target binding sequence and an intramolecularly associated secondary
5 structure 5' to said target binding sequence, wherein at least a portion of said detector
6 sequence is a single stranded tail which is available for hybridization to said target
7 sequence, said detector oligonucleotide having linked thereto,

8 i) a xanthene dye according to claim 1; and
9 ii) a quencher,

10 wherein said detector nucleic acid is in a conformation allowing donor-acceptor
11 energy transfer between said xanthene dye and said quencher when said
12 xanthene dye is excited;

13 (b) extending said hybridized detector oligonucleotide on said target sequence with a
14 polymerase to produce a detector oligonucleotide extension product and separating
15 said detector oligonucleotide extension product from said target sequence;

- 16 (c) hybridizing a primer to said detector oligonucleotide extension product and extending
17 the primer with said polymerase, thereby linearizing said intramolecularly associated
18 secondary structure and producing a change in a fluorescence parameter; and
19 (d) detecting said change in said fluorescence parameter, thereby detecting said target
20 sequence.

1 **36.** (Original) The method according to claim 35, wherein said target sequence is
2 amplified by a method selected from Strand Displacement Amplification, Polymerase Chain
3 reaction, Self Sustained Sequence Replication, Transcription Mediated Amplification, and
4 Nucleic Acid Sequence Based Amplification.

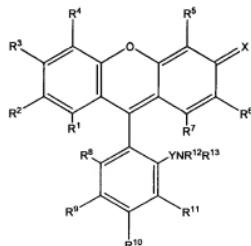
1 **37.** (Original) The method according to claim 35, wherein said secondary structure
2 further comprises a partially or entirely single-stranded restriction endonuclease site.

1 **38.** (Original) The method according to claim 35, wherein a change in fluorescence
2 intensity is detected.

1 **39.** (Original) The method according to claim 38, wherein said change in
2 fluorescence intensity is detected in real-time.

1 **40.** (Original) The method according to claim 35, wherein said intramolecularly
2 associated secondary structure comprises a portion of said target binding sequence.

1 **41.** (Original) A method of preparing a conjugate between a nucleic acid and a
2 xanthene dye having the formula:



4 in which

5 R¹, R², R⁴, R⁵, R⁶, R⁷, R⁸, R⁹, R¹⁰ and R¹¹ are independently selected from substituted or
6 unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or
7 unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or
8 unsubstituted heterocycloalkyl, halogen, H, NO₂, CN and C(Z¹)R¹⁴, NR¹⁵R¹⁶ and
9 Z²R¹⁶;

10 R³ is selected from Z²R¹⁶ and NR¹⁵R¹⁶

11 wherein

12 Z¹ is a member selected from O, S and NH;

13 Z² is a member selected from O and S;

14 R¹⁵ is a member selected from H, substituted or unsubstituted alkyl, and
15 substituted or unsubstituted heteroalkyl;

16 R¹⁶ is selected from H, substituted or unsubstituted alkyl, substituted or
17 unsubstituted heteroalkyl, C(Z³)R¹⁷, and a nitrogen-containing reactive
18 group comprising R¹⁵ and R¹⁶, together with the nitrogen to which they are
19 attached, wherein said reactive group is a member selected from -NHNH₂,
20 -N=C=S and -N=C=O

21 wherein

22 Z³ is a member selected from O, S and NH;

23 R¹⁷ is a member selected from substituted or unsubstituted alkyl,
24 substituted or unsubstituted heteroalkyl, OR¹⁸, and NR¹⁹R²⁰

25 wherein

26 R¹⁸ is a member selected from H, substituted or unsubstituted
27 alkyl, substituted or unsubstituted heteroalkyl, substituted
28 or unsubstituted aryl, substituted or unsubstituted
29 heteroaryl and C(O)R²¹

30 wherein

31 R²¹ is substituted or unsubstituted alkyl or substituted or
32 unsubstituted heteroalkyl;

33 R¹⁹ and R²⁰ are members independently selected from H,
34 substituted or unsubstituted alkyl and substituted or
35 unsubstituted heteroalkyl

36 Y is a member selected from C(O) and S(O)₂;

37 X is a member selected from (NR²²R²³) and (O)

38 wherein

39 R²² and R²³ are members independently selected from H, substituted or
40 unsubstituted alkyl and substituted or unsubstituted heteroalkyl; and

41 R¹² and R¹³ are members independently selected from substituted or unsubstituted alkyl,
42 substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted
43 or unsubstituted aryl and substituted or unsubstituted heteroaryl, with the proviso that at least one
44 of R¹² or R¹³ comprises said nucleic acid.

45 said method comprising:

46 (a) contacting a precursor of said conjugate comprising nucleic acid protecting groups
47 with a mixture of amine and alcohol, thereby removing said protecting groups.